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Ethylene oxide sterilisation—is it safe?

E. H. GILLESPIE[1], J. M. JACKSON[2], AND G. R. OWEN[2]

From the [1]Public Health Laboratory, Northern General Hospital, Sheffield and [2]Department of Chemical Pathology, Sheffield Royal Infirmary, Sheffield, UK

SUMMARY Tests show that ethylene oxide penetrates and can sterilise long narrow tubes in a hospital ethylene oxide steriliser. Residual ethylene oxide levels in plastic tubing after sterilisation have been estimated. Although initially the levels were very high, storage for four days at room temperature reduced them to a safe level. If adequate controls of the sterilising process and storage are carried out, sterilisation by ethylene oxide is considered to be safe for new plastics and clean equipment.

Two essential requirements of sterilisation are that the goods are sterile at the end of the process and that no toxic products are released from the goods to the patient. Unlike the thermocouple readings for heat sterilisation the state of sterilisation by ethylene oxide cannot be assessed from the automatic recording devices alone. Despite the necessary stringent control of temperature, pressure, humidity, and concentration of the gas, the only guarantee of sterility is to perform a biological sterility test using spores of a known culture like *Bacillus subtilis* var *globigii*.

Under efficient supervision by a competent microbiologist these biological tests can guarantee that sterilising conditions have been achieved in the load as long as the packing methods have been supervised to ensure uniform conditions throughout the entire load. Tests of penetration of the gas must be performed to ensure that all the load is adequately exposed to the gas and therefore sterilised. Ethylene oxide may remain as a toxic residue. Testing for such residue on a batch basis is difficult. It is therefore necessary to use a standard procedure which will ensure adequate removal of the toxic products. The investigation reported here studies the safety factor of sterility and levels of toxic residues in order to define procedures that will result in complete confidence in the safety of the material. Although the experiments were originally intended to check the efficiency of the ethylene oxide steriliser the results could be of value to users of similar machines.

Methods and equipment

STERILISATION

Sterilisation was obtained by using a gas mixture (ethylene oxide 15%, carbon dioxide 85%) in a fully automatic 500 litre Sterivit machine (Atesmo Ltd). The temperature of the machine was first raised to 55°C by electric coils, and then a vacuum was produced. After 12 minutes, when it reached 28 inHg absolute (6·76 kPa), water vapour was introduced for a further 12 minutes, giving a relative humidity of 80%. The gas then entered being vapourised over a heater until the pressure in the chamber reached 82 psi (565 kPa gauge pressure) at a final concentration of 1200 mg per litre chamber. Sterilisation was continued for 1 hour. Thereafter a further vacuum of 28 inHg absolute (6·76 kPa) was made to remove the gas, and after 10 minutes sterile air was drawn in for 20 minutes to aerate the load and to accelerate the desorption of absorbed ethylene oxide. If any of the above essential conditions did not occur the machine 'failed safe' by not going on to the next stage. The load was stored for four to seven days for sterility tests or used as required for residual gas analysis.

STERILITY TESTS

Ethylene oxide sensitive papers were attached to each package to check exposure to the gas. Biological test pieces consisting of aluminium strips, each inoculated with 10^6 spores of *B. subtilis* var *globigii*, were placed in every load. After exposure they were tested by the method of Beeby and Whitehouse (1965) by placing the strips in 10 ml of 1/4 strength Ringer solution with Tween 80 in a screw-capped bottle. After shaking, 1 ml of the fluid was transferred to a Petri dish, and a pour plate was made which was incubated aerobically at 37°C for two days. In addition, a more stringent test was made by adding 9 ml of nutrient broth to the remaining fluid in the bottle, and the whole was incubated for seven days and occasionally for 21 days. In some experiments filter paper

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strips impregnated with 10^6 spores of *B. stearothermophilus* were used. Sachets of spores of *B. subtilis* in salt quartz sand (Kristensen, 1970), supplied by the Statens Seruminstitut, Copenhagen, were occasionally used to monitor the conditions in the chamber as these spores are killed only when there is adequate humidification. They were not used routinely.

PENETRATION TESTS

Ethylene oxide penetration was investigated by using a test helix, as described by Line and Pickerill (1973), consisting of a spiral steel tube, 3 mm bore and 4·5 m long (bore/length ratio 1/1500), at the end of which is attached a capsule, 30 mm long and 6 mm diameter, giving an internal volume of 0·85 ml, into which was placed an aluminium spore strip with 10^6 spores of *B. subtilis* var *globigii*. In addition, a modified helix of very fine bore steel tubing (7·5 m long and 0·29 mm internal diameter) was designed and attached to a similar capsule and used as a more difficult test. This gave a bore/length ratio of 1/25 000. Each helix, laid inside a cardboard box to prevent damage, was placed in different positions within the sterilisation chamber in 20 sterilising cycles (Figure).

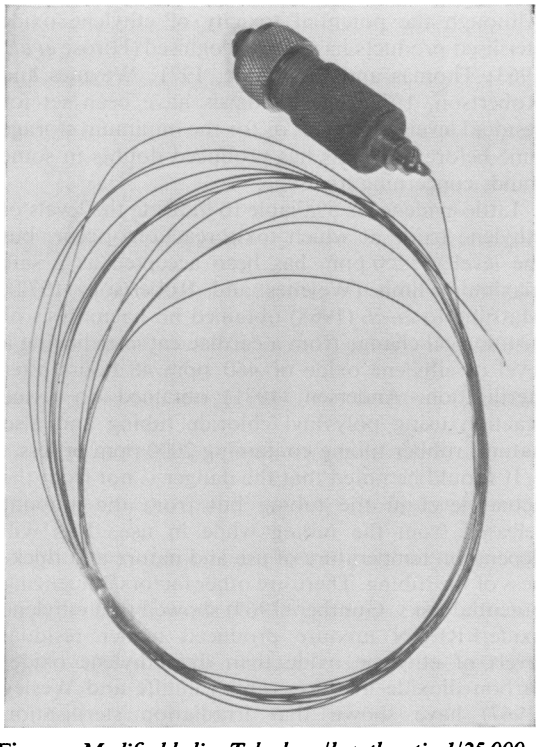

Figure *Modified helix. Tube bore/length ratio 1/25 000. Volume of capsule 0·85 ml.*

DETECTION OF TOXIC RESIDUES

Polythene tubing of dimensions 1·35 mm wall/90 mm diam and 3·00 mm wall/160 mm diam, as employed in heart lung equipment, was wrapped in cotton theatre drapes (Quality No. T 665. Specification DEF 1007), sterilised for 1 hour, and then stored at ambient temperature until used. At a measured time after sterilisation the tubing was immersed in liquid nitrogen and crushed for extraction of the residual gases. A high vacuum distillation extraction adapted from the method of Whitbourne *et al.* (1969) was used.

Quantitative analysis of the extract was performed on a Pye series 104 flame ionisation gas chromatograph 1·5 m long 4 mm id; glass columns were used. For the estimation of ethylene oxide the packing was Porapak Q 80-100 mesh (Chromatography Services) with a column temperature of 90°C, injection temperature of 120°C, and detector temperature of 120°C. The nitrogen flow rate was 45 ml/min.

Results

PENETRATION TESTS

The efficiency of the machine was shown by the fact that, since its installation nine years ago, out of 1625 cycles there have been only 15 bacteriological failures by the plate method, the majority occurring in the first year. These were due to mechanical faults: leaking door seal, water turned off accidentally by the plumber, low gas pressure due to near empty cyliners, faulty gas supplied, and a faulty pressure switch. In all these sterility tests a heavy growth from the spores was obtained. In 15 other sterility tests, failures were obtained by the fluid broth method alone, 10 of these before the humidity of the machine was raised from 65% to 80% two years after its installation. There have been no failures during the last five years, and none occurred during the experiments reported here. To ensure adequate safety in the packs for cardiac surgery, a spore strip was placed routinely in each pack inside a polythene tube which was wrapped in the theatre drapes. All test pieces were sterilised.

The spore strips removed from the two types of helix were all sterile irrespective of length or diameter and indicated a very high penetration ability.

GAS DESORPTION TESTS

(1) Polythene tubing—wall 1·35 mm
(Table 1) —typical results.

Fifteen minutes after sterilisation a very high level of ethylene oxide was found, but this decreased rapidly, and after 48 hours a concentration of 20 ppm was obtained.

(2) Polythene tubing—wall 3·00 mm
(Table 2) —typical results.

Table 1 *Polythene tubing—wall thickness 1·35 mm*

Time post sterilisation (h)	*Conc. ethylene oxide (ppm)*
0·25	10 800
6·25	4 110
23·5	402
48·0	21·2

Table 2 *Polythene tubing—wall thickness 3·0 mm*

Time post sterilisation (h)	*Conc. ethylene oxide (ppm)*
0·2	14 000
53	340
76	90
101·5	18·3

Similar levels to those using 1·35 mm tubing were obtained shortly after sterilisation but, as expected, the desorption was slower, requiring a maximum of 101 hours to reach 18 ppm.

(3) Rubber tubing—wall 1·5 mm (Table 3)

Table 3 *Rubber tubing—wall thickness 1·5 mm*

Time post sterilisation (h)	*Conc. ethylene oxide (ppm)*
0·33	9760
2·5	5720
19·3	578
45·6	44

Results similar to those using 1·35 mm polythene tubing were obtained. For each series of measurements a logarithmic plot of concentration against time gave a straight line, and it was possible to assign a half-life to the desorption process and to calculate the storage time necessary for the limit of 200 ppm to be obtained. Typical half-life values for the 1·35 mm tubing sterilised on different occasions ranged from 4·7 to 7·2 hours. Maximum storage times required to reach 200 ppm were 39 hours for 1·35 mm tubing and 85 hours for 3·00 mm tubing. Table 4 shows a comparison of tubing subjected to an extra period of evacuation and flushing with air compared with tubing sterilised at the same time but with normal treatment. The effect is the equivalent of a two-and-a-half-hour storage at room temperature.

Table 4 *Polythene tubing—wall thickness 1·35 mm*

Time post sterilisation (h)	*Ethylene oxide (ppm)*	
	A	*B*
1·0	10 928	7 798
5·3	3 905	3 740
25·0	42	31

Series A—evacuated normally
Series B—extra 30 minutes' evacuation

Discussion

STERILITY

A potential problem with gas sterilisation is the penetration of long narrow tubes or spaces like cardiac catheters or cystoscopes (Gillespie, 1971, 1973). Line and Pickerill (1973) have shown that their helix test in formaldehyde sterilisation presented a more difficult sterilisation test piece than a cardiac catheter. Using an even more inaccessible 24 ft (7·5 m) long helix of 24 swg (0·46 mm ID) tubing, we have shown that ethylene oxide still has sufficient penetration to kill all test spores in the terminal capsule. The occasional use of sachets of *B. subtilis* in quartz sand has been found to be a necessary but sufficient check of chamber humidity. In earlier experiments the spores in quartz sand had not always been killed, but after the machine had been modified to give a higher humidity (85%), before the experiments reported here, the quartz sand spores have always been killed, so that it was considered unnecessary to use them routinely.

RESIDUAL TOXICITY

Although the potential toxicity of ethylene oxide sterilised products has been recognised (Hirose *et al.*, 1963; Thomas and Longmore, 1971; Weymes and Robertson, 1972), no standards have been set for residual levels of the gas or for the minimum storage time before use. This has produced doubts in some minds concerning its usage.

Little evidence is available to indicate the levels of ethylene oxide at which toxic reactions occur, but the level of 200 ppm has been accepted as a safe maximum limit (Weymes and Robertson, 1972). Matsumoto *et al.* (1968) obtained no haemolysis or histological change from a cardiac catheter having a level of ethylene oxide of 460 ppm 48 hours after sterilisation. Andersen (1971) obtained no tissue reaction using polyvinyl chloride tubing and also natural rubber tubing containing 2000 ppm or less.

It should be noted that the danger is not from the actual level in the tubing but from the amount released from the tubing while in use. This will depend on temperature of use and nature and thickness of the tubing. There are other factors influencing potential risks. Gunther (1969) showed that ethylene oxide/FREON mixture produced higher residual levels of ethylene oxide than did ethylene oxide/carbon dioxide mixtures, and Cunliffe and Wesley (1967) have shown that irradiation sterilisation before ethylene oxide sterilisation increases the level of 2-chloroethanol in PVC tubing.

We consider the evidence indicates that an upper limit of 200 ppm is a realistic safe limit, and we have shown that levels lower than this are easily obtained on storage at room temperature. Although the level immediately after sterilisation is very high, about 10 000 ppm, it falls to nearly 20 ppm in two days in 1·35 mm tubing and in four days in 3 mm tubing. Extended aeration of the sample in the chamber was not found to save a significant amount of time.

Although not tested by us, the desorption is encouraged by storage at higher temperatures, and significant time savings can be obtained (Gunther, 1969). As a result of our experiments we calculated that if aeration is performed at warm room temperature and storage is continued for at least four days there should be no risk of toxicity. In an emergency, even at 48 hours normal cardiac tubing will also be safe. The present recommendation that storage must be for a minimum of four days is a useful safeguard for all routine sterilisation so that there should be no risk from ethylene oxide. By this time the results of the bacteriological spore tests will also be available.

Conclusions

If ethylene oxide sterilisation is carried out at hospital level it can be considered safe in terms of both sterility and freedom from toxicity if it is correctly controlled. Although one cannot routinely test plastics for toxic residues, one should make use of established methods which will reduce the potential hazards, as, for example, the use of carbon dioxide/ethylene oxide mixtures, close control of the sterilisation process using a short exposure to the gas at a high pressure, and storage of the products at a warm room temperature (20-30°C) for a minimum of four days after sterilisation. It must be recorded that all goods for gas sterilisation must be clean, and plastics should preferably be used only as disposables. As with recommendations for heat sterilisation, all machines should be fully automatic with 'fail safe' controls, and the process from packing to the sterility tests must be under the control of a competent bacteriologist fully aware of the potential dangers.

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Requests for reprints to: Dr G. R. Owen, Department of Chemical Pathology, Royal Infirmary, Sheffield S6 3DA, UK.